CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Famoxadone

Chemical Code # 5878, Tolerance # 52960
SB 950 # NA.

9 March 2004

I. DATA GAP STATUS

Combined, rat:	No data gap, no adverse effects
Chronic toxicity, dog:	No data gap, possible adverse effects
Chronic toxicity, monkey	No data gap, possible adverse effects
Oncogenicity, mouse:	No data gap, no adverse effects
Reproduction, rat:	No data gap, no adverse effects
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, possible adverse effect
DNA damage:	No data gap, possible adverse effect
Neurotoxicity:	No data gap, no adverse effect

Toxicology one-liners are attached.

All record numbers through 207581 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study in review.
File name: T040309
Prepared by Green, 9 March 2004

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

**52960-0047 207349 MacKenzie, S. A., "Combined chronic toxicity/oncogenicity study with DPX-JE874-221: Two-year feeding study in rats," Haskell Laboratory, Newark, DE, 4/15/96. Laboratory Study #: DuPont HLR 527-95. Sixty-two CRL:CD®BR rats/sex/group were dosed in diet with famoxadone (97.4% purity) at 0, 10, 40, 200, or 400 ppm in a combined chronic/oncogenicity study. Lifetime study rats had mean dose levels of 0.42, 1.62, 8.4, or 16.8 mg/kg/day for males, and 0.53, 2.15, 10.7, or 23.0 mg/kg/day for females. Lifetime study females were maintained for 24 months, whereas corresponding males were sacrificed at 23 months as mortality approached 75% (not dose-related). Survival was sufficient for a valid study. Additional rats assigned at the same treatment levels included: 10/sex/group for 1-year interim sacrifice, 5/sex/group/interval for biochemical examinations of liver homogenates (days 16-17 for the first sampling, and days 359-360 for the second sampling) to assess peroxisomal fractions for beta oxidation activity and microsomal fractions for cytochrome P-450 content, and 5/sex/group/interval on a comparable schedule for liver cell proliferation evaluations following 3 days of pre-treatment with BrdU. NOEL = 40 ppm, based on reduced RBC counts and reduced Hb levels in females, and slightly increased hepatocellular hypertrophy in males. High dose females had significantly reduced body weights at 2-year term (14% decrement), whereas high dose males were marginally lower than controls in body weight (4% at term). There were no sustained food consumption decrements nor treatment-related clinical signs or mortality. Hematology, which was assessed at 5 intervals during the study, revealed consistent, statistically significant decrements in RBC counts, Hb, and HCT in 400 ppm females. RBC counts were also depressed at most assay times in 400 ppm males and 200 ppm females. These females also generally had reduced Hb. Slight (not statistically significant) incidences or increases in pigmented Kupffer cells, splenic extramedullary hematopoiesis, or bone marrow hyperplasia also suggested hemolytic responses, as did statistically significantly increases of grossly enlarged spleens at 400 ppm in both sexes. The liver was also a target organ. Hepatocellular hypertrophy was significantly elevated at 400 ppm in both sexes at interim and term sacrifices. Lifetime study hypertrophy incidences in males were 0, 0, 0, 3, and 19 (N = 62), indicating a mild treatment response at 200 ppm also. Hepatocellular focal degeneration and eosinophilic foci of alteration in liver were elevated at 400 ppm (probably treatment-related but usually not statistically significant). Peroxisomal β-oxidation and cytochrome P-450 content assays suggested slight hepatocellular induction in 400 males and females, respectively. Hepatocyte labeling indices suggested an increase after short term exposure in males only, consistent with at least a transient increased hepatocyte turnover. There was no associated liver neoplasia. Investigators noted a trend for increased testicular interstitial cell tumors (incidences of 0, 0, 1, 1, and 3 in controls through increasing dose groups), however these incidences were neither statistically significant nor out of historical control range. No neoplasia is inferred by investigators nor by this reviewer for any tissues. Acceptable, with no adverse effects. Aldous, Feb. 3, 2004.

52960-0048 207350 [Ocular histopathology supplemental to: 52960-0047 207349]. The original review did not consider there to be ocular histopathology, although there was a non-significant increase in lifetime study males (3 controls vs. 7 high dose males were diagnosed with cataracts in the original examination). The supplementary data (including re-examination of all eyes of interim and lifetime study males) also do not indicate treatment responses at either time period. (No change in study status: acceptable, no adverse effects). Aldous, 12/29/03.

52960-0049 207351 [Dose justification supplemental to: 52960-0047 207349]. This brief report was submitted to address adequacy of the highest dose level used in the combined study. Outcomes of the primary study and of the subchronic study and 28-day subacute study in rats

(also reviewed by DPR) as well as the results of the primary combined study were presented as justification of dose levels. Dose level adequacy was not challenged in the original DPR review. This supplement does not require a DPR review, since there is no essential unique information in this record. Aldous, 12/29/03.

CHRONIC TOXICITY, RAT

See "Combined" Rat, above.

CHRONIC TOXICITY, DOG

****52960-0043 207345** Mertens, J. J. W. M., "Chronic toxicity study with DPX-JE874-221: One year feeding study in dogs," Haskell Laboratory, Newark, DE, May 6, 1996. Laboratory Study #: DuPont HLO 820-95. Project No. WIL-189013. Four beagles/sex/group were dosed in diet with Famoxadone (adjusted for purity of 97.4%) for one year at 0, 10, 20, 40, or 300 ppm. Estimated achieved dose levels were 0.3, 0.6, 1.2, and 8.8 mg/kg/day for males, and 0.3, 0.6, 1.2, and 9.3 mg/kg/day for females. An additional 4/sex were dosed at 300 ppm for 3 months, then taken off treatment for 9 months ("recovery group"). NOTE: The previous 90-day study (DuPont HLO 500-94, DPR Document No. 52960-0038), had found ocular lesions in several 300-600 ppm dogs and in one 40 ppm female. This influenced dose level selection for the chronic study and prompted inclusion of ophthalmological examinations eight times during this chronic study. The subchronic study also revealed myotonic twitches in several 600 to 1000 ppm dogs, hence a neurological examination was conducted at week 51 of the chronic study, which assessed changes in appearance, behavior, locomotion, and possible changes in a large variety of reflexes. NOEL for the chronic study = 40 ppm. Key findings in the chronic study at 300 ppm were lenticular lesions: posterior subcapsular cataracts (normally bilateral and commonly appearing by 3 months of treatment), and equatorial cataracts (always bilateral and first appearing after at least 6 months of treatment). These cataracts were generally confirmed as lenticular degeneration at histopathology, and are considered "possible adverse effects." All recovery group dogs displayed bilateral posterior subcapsular cataracts: in 7/8 dogs the first observation was at 3 months. Four of these dogs showed some regression of cataracts: in 3 of them there was at least unilateral disappearance of cataracts by the end of the study. Equatorial cataracts never appeared in recovery group dogs. There was no regression of either cataract type in 300 ppm (continuous) dogs. The only other plausible treatment effect was elevated platelet counts: statistically significant at 3 months in 300 ppm females and in 300 ppm recovery males (still on treatment at time of assay). There were no associated alterations of RBC parameters or reticulocyte counts at any dose level. The regression of several posterior subcapsular cataracts in several 300 ppm/recovery dogs is a factor to consider in chronic effects endpoint selection. The neurological examination found no treatment effects. This study is **acceptable**, with some deficiencies noted in the review. Aldous, 2/23/04.

CHRONIC TOXICITY, MONKEY

****52960-0044, 0051 207346**, **207353,** "52-Week Oral Gavage Toxicity Study with DPX-JE874-221 in Cynomolgus Monkeys (Final Report)", (Kevin D. Williams, Corning Hazleton Inc., Madison, Wisconsin, Report No. DuPont HLO-1997-00583, 17 October 1997). 4 cynomolgus monkeys per sex per group received DPX-JE874-221 Technical (97.4% famoxadone) by oral gavage at 0 (0.5% Tween® 80 in reverse osmosis (RO) water), 1, 100, and 1000 mg/kg/day for 52 weeks. One male at 1 mg/kg/day was sacrificed due to poor health (attributed to dosing error) on day 14. One high dose female died on day 77, cause undetermined. All other animals survived to scheduled sacrifice. No apparent treatment-related effects on behavior, bodyweight, food consumption, ophthalmology, clinical chemistry, urinalysis, or gross pathology. Red blood cell count, hemoglobin, and hematocrit were reduced for both sexes at 1000 mg/kg/day at all sampling times during the treatment period. Reductions in the latter two attained statistical significance. Secondary to the anemia, histopathology revealed mild sinus dilatation of the spleen and minimal

to mild increases in blood breakdown pigments in spleen, liver, and kidney for both sexes at 1000 mg/kg/day. Chronic NOEL = 100 mg/kg/day (anemia). The eyes were given extensive histologicla examination with no treatment-related findings. Possible adverse effect: anemia (reduced red blood cell count, hemoglobin, and hematocrit) at 1000 mg/kg/day. Record 207353 contains a summary and discussion of the hematologic effects of famoxadone in mammal (rodent, dog, and primate) studies. Acceptable. (Green and Gee, 1/22/04).

ONCOGENICITY, RAT

See “Combined” Rat, above.

ONCOGENICITY, MOUSE

**52960-0045 207347 MacKenzie, S. A., “Oncogenicity study with DPX-JE874-221: Eighteen-month feeding study in mice,” Haskell Laboratory, Newark, DE, 3/29/96. Laboratory Study #: DuPont HLR 526-95. Sixty CRL:CD1®(ICR)BR mice/sex/group were dosed in diet for 18 months with famoxadone (97.4% purity) in an oncogenicity study at 0, 5, 50, 700, or 2000 ppm, equivalent to 0.7, 6.8, 96 or 274 mg/kg/day in treated males, and 1.0, 9.8, 130, or 392 mg/kg/day in treated females. Additional mice assigned at the same treatment levels included 5/sex/group/interval for immunohistochemical evaluations of liver cell proliferation following 3 days of pre-treatment with BrdU. These assessments were performed after 2 weeks and 9 months of treatment. Another 5/sex/group per interval were designated for biochemical examinations of liver homogenates (also after 2 weeks and 9 months) to assess peroxisomal fractions for β-oxidation activity and microsomal fractions for cytochrome P-450 content, respectively. Chronic NOEL = 50 ppm, based on eosinophilic foci of cellular alteration in livers of males, plus numerous adaptive changes at 700 and 2000 ppm, including centrilobular hypertrophy (both sexes), panlobar hypertrophy in females, elevated liver weights in both sexes, and elevated peroxisomal β-oxidation rate and elevated cytochrome P-450 content in liver homogenates of both sexes. Panlobular hepatocellular hypertrophy was also observed in one 50 ppm female, and female liver homogenates yielded significantly increased cytochrome P-450 content at 50 ppm on day 14. Both of the latter are also considered to be reversible responses to liver induction, and do not apply to the chronic NOEL. Evidences of liver toxicity at 2000 ppm included a slight increase in apoptosis in females, a significant increase in incidence of focal necrosis in males, and significantly increased Kupffer cell pigment in both sexes. Such pigment contained primarily lipofuscin, indicative of ongoing toxicity. Pigment contained very little hemosiderin, suggesting that hemolysis was much less evident in the mouse compared to other species. There was a general increase in amyloid accumulation in multiple tissues in 2000 ppm females, considered by investigators to be an exacerbation of normal aging processes. Acceptable, with no adverse effects. Aldous, 2/26/04.

52960-0046 207348 Cox, L. R. [Supplement No. 2 to oncogenicity study, 52960-0045 207347, above]. The high dose utilized in the oncogenicity study was 2000 ppm for both sexes. This short record acknowledges that it is probable that a high dose of 3500 ppm would have been survivable in males. At 2000 ppm, males showed limited hepatocellular necrosis and some lipofuscin accumulation. In females, however, eight of the 2000 ppm mice died of amyloidosis, vs. 2 or 3 in each of the other groups. It appears likely that amyloidosis would have severely limited survival of females if the high dose had been raised to 3500 ppm. Investigators contend that 2000 ppm achieved an MTD for females, and was close enough to an MTD in males for a valid study. The report included tables showing marked increases in liver-associated serum chemistry effects. There was significantly elevated alkaline phosphatase, alanine aminotransferase, and sorbitol dehydrogenase in males administered 3500 ppm for 2 weeks. In subsequent studies with samplings at 2 weeks and 4 weeks, dose levels of 2000 ppm and above quite reliably elevated alkaline phosphatase and sorbitol dehydrogenase, with some significant elevations for alkaline phosphatase at 500 ppm. Liver diffuse fatty change was noted in the 90-day subchronic mouse

dietary study at 3500 to 7000 ppm. Further, peroxisomal β-oxidation rate was significantly elevated at 1000 ppm and above after 28 days of exposure, and cytochrome P-450 content was statistically significantly elevated after 28 days at 100 ppm and above (although increases above 150% of control were limited to 500 ppm and above). The DPR review had accepted the oncogenicity study on the basis of its own dose-response. The data and perspectives from this record validate that choice. Aldous, 2/26/04 (no worksheet).

REPRODUCTION, RAT

**52960-0057 207359 Kreckmann, K. H., "Reproductive and fertility effects with DPX-JE874-221: multigeneration reproduction study in rats," Haskell Laboratory, Newark, DE, 10/31/95. Laboratory Study #: DuPont HLR 238-95. Thirty CRL:CD®BR rats/sex/group were dosed in diet with DPX-JE874-221 (Famoxadone, purity 97.4%) continuously at dose levels of 0, 20, 200, or 800 ppm in a standard reproduction study, with one mating period per generation. Pre-mating periods were 70 days for the F0 generation, and 105 days for the F1 generation. Estimated mean pre-mating exposures were 1.14, 11.3, and 45 mg/kg/day for F0 males and 1.45, 14.2, and 53 mg/kg/day for F0 females. Exposures for F1 rats, which began treatment upon weaning, were 1.48, 14.8, and 62 mg/kg/day (M) and 1.80, 17.5, and 72 mg/kg/day (F). To assess liver toxicity and functional changes, 10 fasted rats/sex/group were bled near to the end of each pre-mating period for clinical chemistry test series, which included several liver-associated enzymes and biomolecules. Also, liver homogenates were prepared from 5 rats/sex/group/generation for peroxisomal β-oxidation measurements. Parental systemic toxicity LOEL = 200 ppm, based on minor elevations of cholesterol at the end of the pre-mating period. A NOEL of 50 ppm from the subchronic study (Record No. 207336, which also found minor elevations of cholesterol after similar duration, using same strain of rats at the same laboratory as the present study) is appropriate for parental toxicity in this study. Parental reproductive effects NOEL = 800 ppm (HTD: no reproductive responses). Offspring viability and growth NOEL = 200 ppm (7 to 10% body weight decrement at weaning of 800 ppm pups). Other parental effects at 800 ppm included 7-12% body weight decrements with associated small reductions in food consumption, consistent elevations in circulating enzymes associated with hepatocellular toxicity in males (alkaline phosphatase, alanine aminotransferase, asparate aminotransferase, and sorbitol dehydrogenase: each of these significantly elevated, $p < 0.05$, at end of pre-mating periods of both generations), elevated cholesterol and reduced triglycerides (generally statistically significant in both sexes of both generations), slightly elevated bilirubin (significant in males only), and slightly elevated BUN in both sexes. Liver toxicity and functional changes are inferred from clinical chemistry, but were not assessed microscopically in this study (see subchronic study for relevant findings). Bilirubin elevations are consistent with modest hemolysis as indicated in the subchronic study (hematology was not performed in this study). Circulating creatinine was also assayed, and showed no response, thus possible indications of kidney toxicity (i.e. elevated BUN) were not substantiated. Liver peroxisomal β-oxidation was significantly elevated in both sexes of both generations at 800 ppm. Study is acceptable, with some deficiencies noted in worksheet. Recent design features recommended in 1998 EPA guidelines, such as sperm motility and morphology analyses and quantitative evaluations of ovarian primordial follicles, were not included in this study design. No adverse effects. Aldous, Feb. 3, 2004.

TERATOLOGY, RAT

**52960-0053, 0054 207355, 207356, "Developmental Toxicity of DPX-JE874-221 in Rats", (Susan M. Murray, E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, Report No. DuPont HLR 375-94, 17 October 1994). 25 mated female Crl:CD® BR rats per group received DPX-JE874-221 (97.4% famoxadone) at 0 (water plus 0.5% Tween 80), 125, 250, 500, and 1000 mg/kg/day on gestation days 7 through 16. The 1000 mg/kg dose was discussed as the maximum achievable in 10 ml/kg. Statistically significant reductions in maternal bodyweight gain and food consumption were recorded for

gestation days 7-9 at 500 and 1000 mg/kg. Final weights on day 22 were comparable. Maternal NOEL = 250 mg/kg/day. No treatment related effects on any reproductive parameter were indicated. No teratogenicity. Developmental NOEL = 1000 mg/kg/day. Acceptable. (Green and Gee, 1/21/04).

TERATOLOGY, RABBIT

**52960-0055, 0056 207357, 207358, "Developmental Toxicity of DPX-JE874-221 in Rabbits, Revision No. 1" (Susan Murray Munley, E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, Report No. DuPont HLR 479-94, original report completed 16 November 1994; Revision No. 1 completed 5 April 1999, supplement No. 1 July 23, 1999). 20 time-mated female Hra:(NZW)SPF rabbits per group received DPX-JE874-221 Technical (97.4% famoxadone) by oral gavage at 0 (water plus 0.5% Tween 80), 100, 350, and 1000 mg/kg/day on gestation days 7-19 in 10 ml/kg. 4 females aborted (statistically significant by trend test but not Fishers' Exact) at 1000 mg/kg/day during gestation days 19-23. All four animals exhibited severe decrement in bodyweight gain and food consumption. They also had tan stools. One of 2 females that aborted at 100 mg/kg/day also showed similar signs. There were no abortions at 1000 mg/kg in the 20 does in the pilot study. In the absence of dose-dependent increases, the study author concluded that post-mortem findings indicated gastrointestinal impaction (data not included), a stress-related response to being gavaged with a large volume of a thick, maximally-concentrated dosing suspension as the cause rather than systemic compound toxicity. Maternal NOEL = 1000 mg/kg/day. No compound-related effects on fetal development were indicated. Developmental NOEL = 1000 mg/kg/day. Acceptable. (Green and Gee, 1/22/04).

GENE MUTATION

**52960-0058 207360, "Mutagenicity Testing of DPX-JE874-221 in the *Salmonella Typhimurium* and *Escherichia Coli* Plate Incorporation Assay" (Karin S. Bentley, E. I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, Haskell Laboratory Report No. 707-94, 14 February 1995). Triplicate cultures of *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535 and *Escherichia coli* strain WP2 uvrA (pKM101) were exposed to DPX-JE874-221 Technical (97.4% famoxadone), in the presence and absence of S9 rat liver fraction, at 0 (DMSO), 10, 50, 100, 500, 1000, 2500, and 5000 µg/plate for 48 hours in two separate trials. No increase in the reversion rate. Positive controls were functional. Acceptable. (Green and Gee, 1/20/04).

**52960-0059 207361, "Famoxadone Technical (DPX-JE874): *In Vitro* Mammalian Cell Gene Mutation Test (CHO/HGPRT)", (Maria A. Cifone, Covance Laboratories Inc., Vienna, VA., Covance Study No. 20129-0-435 OECD, DuPont Report No. 1821, 10 March 1999). Chinese hamster ovary cells (CHO-K1-BH_4) were treated in duplicate, in the presence and absence of S9 rat liver fraction, at DPX-JE874 (97.26% famoxadone) concentrations of 0 (1% DMSO), 75, 100, 150, 175, 200, 250, 300, 350, 400, 450, 500, or 600 µg/ml for 4 hours. Cells were plated at 4 x 10^6 cells per T-75 (75 cm^2) tissue culture flask on the day before treatment. After treatment, cells were replated at 1.5 x 10^6 cells into each of two 150 mm dishes (for mutant expression) and 200 cells into each of three 60 mm dishes (for cytotoxicity) followed by incubation for seven days. No increase in the induction of forward mutations at the HGPRT locus was indicated. Positive controls were functional. Acceptable. (Green and Gee, 1/20/04).

CHROMOSOME EFFECTS

****52960-0060 207362**, "*In Vitro* Evaluation of DPX-JE874-221 for Chromosome Aberrations in Human Lymphocytes" (Kathy M. Gerber, E.I. du Pont de Nemours and Company; Haskell Laboratory for Toxicology and Industrial Medicine; Newark, Delaware; Report No. HLR 25-95, 6 April 1995). Human (male and female) whole blood cultures (48 hours with PHA) (0.4 ml) were

exposed to DPX-JE874-221 (97.4%), in the presence and absence of S9 activation, at 0 (DMSO), 10, 15, 20, 25, or 30 μg/ml for 3 hours. Cells were then rinsed, recultured with 5-bromodeoxyuridine, incubated for 18 to 23 hours, then mounted on slides and evaluated (50 cells per person, two trials). An increase (statistically significant) in chromosomal aberrations was indicated in the absence of S9 activation. Acceptable. (Green and Gee, 1/20/04).

**52960-0061 207363, "Mouse Bone Marrow Micronucleus Assay of DPX-JE874-221" (Jimmy R. Kuykendall, E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, Report No. HLR 96-94, 2 August 1994). 5 or 6 (high dose) Crl:CD®-1(ICR)BR mice per sex per group received a single oral gavage dose of DPX-JE874-221 Technical (97.4% famoxadone) at 0 (Corn oil), 1250, 2500, and 5000 mg/kg followed by bone marrow sampling 24 hours later. Vehicle control and high dose animals were also sampled 48 and 72 hours post-dosing. 3 slides per animal were prepared. 2000 polychromatic erythrocytes were scored per animal. No increase in the frequency of micronucleated polychromatic erythrocytes was indicated. Acceptable. (Green and Gee, 1/21/04).

DNA DAMAGE

**52960-0062 207364, "DPX-JE874-221: Measurement of Unscheduled DNA Synthesis in Rat Liver Using an *In Vivo/In Vitro* Procedure", (M. Fellows, Covance Laboratories Limited, North Yorkshire, England, Report No. DuPont HLO-1998-01212, 30 April 1998, Revision no. 1, 13 May 1998, and Revision no. 2, 21 September 1998). Five male Sprague Dawley Crl:CD®BR rats per group were exposed (oral gavage) to DPX-JE874-221 Technical (97.4% famoxadone) at 0 (corn oil), 800, and 2000 mg/kg. Animals were sacrificed and hepatocytes sampled 2 to 4 hours and 14 to 16 hours post-dosing. Hepatocytes from 3 per group were analized. No induction of unscheduled DNA synthesis. Acceptable. (Green and Gee, 2/24/04).

**52960-0063 207365, "Famoxadone Technical (DPX-JE874): Unscheduled DNA Synthesis in Mammalian Cells *In Vitro,* Revision No. 1", (Maria A. Cifone, Covance Laboratories, Inc., Vienna, VA., Covance Study No. 20129-0-447R, DuPont Report No. 1822, the original report was completed 10 March 1999. Revision No. 1 was completed 1 April 1999). Hepatocytes from male Crl:CD® (SB)BR rats were exposed in quintuplicate cultures (2 for cytoxicity) to DPX-JE874-221 (97.28% famoxadone) at 0.100, 0.250, 0.500, 1.00, 2.50, and 5.00 μg/ml for 18.9 and 19.16 hours in 2 trials. 50 cells per coverslip were evaluated by autoradiography. No increase in unscheduled DNA synthesis. Acceptable. (Green and Gee, 2/24/04).

**52960-0200 207581, "Assessment of DPX-JE874-133 in the *In Vitro* Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes", (Kathy M. Gerber, E. I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE., Report No. DuPont HLR 737-93, 5 August 1994). Quadruplicate cultures of male Crl:CD®BR rat hepatocytes were exposed to DPX-JE874-133 (technical) (97.7% cymoxanil) at 0 (DMSO), 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, or 10.0 μg/ml for 18 hours in 3 autoradiographic trials. 25 cells from each culture, two cultures from each concentration, were scored using an automatic colony counter interfaced via a remote camera to a microscope. DPX-JE874-133 increased unscheduled DNA synthesis in all three trials but was not clearly dependent on concentration of the a.i. Acceptable. (Green and Gee, 2/24/04).

NEUROTOXICITY

Acute Neurotoxicity

0029; 207330; "Acute Neurotoxicity Study of DPX-JE874-221 in Rat" (Malley, L.A., E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, Haskell Laboratory Report No. 513-94, 5/24/95). 818. DPX-JE874-221 Technical (Batch No. DPX-JE874-221, purity = 97.4%), suspended in 0.5% Tween 80/99.5% deionized water, was administered as a single gavage dose to 12 Crl:CD®BR rats per sex per dose at dose

levels of 0 (vehicle only), 500, 1000, and 2000 mg/kg. No mortalities occurred. Treatment-related reduced body gain and food consumption in the Day 1-2 interval were observed in males at 2000 mg/kg. A treatment-related higher incidence of palpebral closure during Day 1 FOB home cage and open field arena observations was observed in males at 2000 mg/kg. No treatment-related effects were observed during FOB assessments conducted on Days 8 and 15. Motor activity assessments revealed no treatment-related effects on Days 1, 8, and 15. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M) = 1000 mg/kg (based on reduced body weight gain and food consumption during the Day 1-2 interval and a higher incidence of palpebral closure during Day 1 FOB home cage and open field arena observations), NOEL (F) = 2000 mg/kg (no effects observed at the highest dose tested). **Acceptable.** (Corlett and Leung, 03/15/04)

Subchronic Rat Oral Neurotoxicity Study

0042; 207344; "Subchronic Oral Neurotoxicity Study of DPX-JE874-221 in Rat" (Malley, L.A., E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, Haskell Laboratory Report No. 239-95, 12/04/95). 827. DPX-JE874-221 Technical (Batch No. DPX-JE874-221, purity = 97.4%) was admixed to the feed and fed to 12 Crl:CD®BR rats per sex per dose at dose levels of 0 (untreated diet), 50, 200, or 800 ppm (0, 2.90, 11.7, 46.9 mg/kg/day, respectively for males and 0, 3.70, 14.4, 59.3 mg/kg/day, respectively for females) 7 days per week for 13 weeks. No mortalities occurred. No treatment-related clinical signs were observed. A treatment-related decrease in mean body weight gain was observed in males at 800 ppm from Day 0 to Day 7 and in females at 800 ppm from Day 0 to Day 91. A treatment-related decrease in mean daily food consumption from Day 0 to Day 7 was observed in males and females at 800 ppm. No treatment-related effects were observed during FOB assessments. Motor activity assessments revealed no treatment-related effects. Macroscopic and neuropathological examinations revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M)= 11.7 mg/kg/day (200 ppm) and NOEL (F) = 14.4 mg/kg/day (200 ppm) based on decreases in body weight gain and food consumption. **Acceptable.** (Corlett and Leung, 03/22/04)

METABOLISM

52960-0068, 0069 207370, 207371, "Absorption, Excretion, Distribution and Metabolism of [^{14}C]DPX-JE874 in Rats",** (Michael C. Savides, *et al*., Ricerca, Inc., Department of Toxicology and Animal Metabolism, Painesville, OH., Ricerca Report No. 5492-92-0422-AM-001, DuPont Study No. AMR 2440-92, 27 January 1995). Ten groups of 4 or 5 Crl:CD/BR (Sprague-Dawley) albino rats per sex received a single oral gavage dose of [^{14}C-PA]DPX-JE874] at 5 or 100 mg/kg. One group (G) of 5 per sex had been exposed (oral gavage) to non-radiolabelled DPX-JE874 for fourteen consecutive days prior to the radiolabelled dose. Two other groups (B (4/sex) and E (5/sex)) received a single dose (oral gavage) of [^{14}C-POP]DPX-JE874 at 100 mg/kg. <u>Groups A, B, and C</u> The absorption half-lives of [^{14}C-PA]DPX-JE874 in whole blood and plasma increased from 0.8 - 1.2 hours to 3.5 - 7.1 hours as the dose increased from 5 to 100 mg/kg. Absorption half-lives of [^{14}C-POP]DPX-JE874 at 100 mg/kg were 0.4 to 1.4 hours. Elimination half-lives were 2 to 3 fold slower in whole blood compared to plasma with [^{14}C-PA]DPX-JE874 (indication of binding to red blood cells). No indication of binding with [^{14}C-POP]DPX-JE874. <u>Groups D, E, F, G</u> No accumulation of [^{14}C-PA]DPX-JE874 residues in organs and tissues was observed at 5 and 100 mg/kg 120 hours post-treatment. [^{14}C-POP]DPX-JE874 treated animals (Group E, 100 mg/kg) showed highest radioactivity in fat (< 2 ppm). Gonads, uterus, adrenals, and bone marrow also contained slightly increased levels of ^{14}C-residues (< 2 ppm) (possibly associated with body fat adhering to the tissues). > 75% of administered radiolabel was excreted in feces and less than 10% in urine during 24 hours post-dosing. There was no significant difference in the elimination profile between single (D, E, and F) and multiple (G) dosings, between sexes, nor between [^{14}C-PA]DPX-JE874 and [^{14}C-POP]DPX-JE874. Three radioactive components were observed in feces of both [^{14}C-PA] and [^{14}C-POP] treated animals. Unmetabolized ^{14}C-DPX-JE874 was the

major component. The other two were monohydroxylated (IN-KZ007) and di para hydroxylated (IN-KZ534) DPX-JE874. One major radioactive component (a sulfate conjugate) was observed in urine of [^{14}C-POP] treated animals. The primary metabolite in urine from [^{14}C-PA] treated animals coincided with 4-acetoxyaniline (HPLC). Groups H and I Liver and fat were the two primary tissues for distribution of [^{14}C-PA]DPX-JE874 residues at 5 hours (5 mg/kg) and 14 hours (100 mg/kg) post-dosing. At 36 hours (5 mg/kg) and 48 hours (100 mg/kg), liver was the only tissue containing slightly elevated residues. Record 207371 in 52960-0069 Isomeric composition of recovered [^{14}C]DPX-JE874 from rat feces was compared to [^{14}C]DPX-JE874 in the original dosing material. Stereoselective metabolism was minimal. Acceptable with 207372. (Green and Gee, 2/25/04).

**52960-0070 207372, "Biliary Excretion of [^{14}C]DPX-JE874 in Rats", (Michael C. Savides, *et al*., Toxicology and Animal Metabolism, Ricerca, Inc., Painesville, OH., Ricerca Document No. 6667-95-0277-AM-001, DuPont Study No. AMR 3707-95, 15 January 1997). 7 rats per sex received a single oral gavage dose of [^{14}C-PA]DPX-JE874 or [^{14}C-POP]DPX-JE874 at 5 mg/kg. The animals had biliary and duodenal cannulae surgically implanted 3 days prior to treatment. Bile was collected continuously and sampled 1, 3, 6, 10, 16, 24, 36, and 48 hours post-dosing. Urine and feces were collected 12, 24, and 48 hours after dosing. After the final urine and feces collection, cage washes were collected and analyzed. Blood was collected from all animals at termination (48 hours post-treatment). Carcasses were homogenized and analyzed for radioactivity. 30% to 39% of administered radiolabel was excreted in bile 1 to 10 hours post-dosing. Higher amounts of [^{14}C-POP]DPX-JE874 (39%) than of [^{14}C-PA]DPX-JE874 (31%) were excreted in males. The average urinary excretion of radiolabel was from 2% to 6% of administered dose. 56% to 65% of administered dose was excreted in feces. 0.22% and 0.31% of administered dose was found in blood of [^{14}C-PA]DPX-JE874 treated males and females respectively at termination. 0.03% was found in blood of both males and females treated with [^{14}C-POP]DPX-JE874. The average amount of radiolabel in carcasses ranged from 0.4% to 3.0%. 2 animals per group were used for analysis of feces and bile. Fecal extracts contained only unmetabolized DPX-JE874 (amounts ranged from 26% to 58% of administered dose). DPX-JE874 was not detected in bile samples. Bile was treated with β-glucuronidase/sulfatase to release glucuronide and sulfate conjugates to the corresponding non-conjugated metabolites. IN-KZ007 (5-[4-(4-hydroxyphenoxy)phenyl]-5-methyl-3-(phenylamino)-2,4-oxazolidinedione), IN-KZ532 (3-[(4-hydroxyphenyl)amino]-5-methyl-5-(4-phenoxyphenyl)-2,4-oxazolidinedione), IN-KZ534 (5-[4-(4-hydroxyphenoxy)phenyl]-3-[(4-hydroxyphenyl)amino]-5-methyl-2,4-oxazolidinedione), IN-ML815 (α-hydroxy-4-(4-hydroxyphenoxy)-α-methylbenzeneacetic acid 2- phenylhydrazide), and catechol (1,2-dihydroxybenzene) were observed as products of enzyme treatment in bile of [^{14}C-PA]DPX-JE874 treated animals. For the [^{14}C-POP]DPX-JE874 treated animals, IN-KZ007, IN-KZ532, IN-KZ000 (5-[4-(4-hydroxyphenoxy)phenyl]-5-methyl-2,4-oxazolidinedione), IN-KZ534, IN-ML815, and IN-ML436 (α-hydroxy-4-(4-hydroxyphenoxy)-α-methyl-4-phenoxybenzeneacetic acid) were observed. Conjugates of IN-KZ007 and catechol and conjugates of IN-KZ007 and IN-ML436 were the major metablolites in bile of [^{14}C-PA]DPX-JE874 and [^{14}C-POP]DPX-JE874 treated animals, respectively. A proposed metabolic pathway (schematic) was included (Figure 21, page 79). Acceptable in conjunction with 207370. (Green and Gee, 2/24/04).

*52960-0071, 0072 207418, 207419, "Absorption, Metabolism, and Excretion of [^{14}C-PA]DPX-JE874 Following Single Oral Doses in Male Beagle Dogs", (Frederic W. Thalacker, Corning Hazleton Inc., Madison, WI., Report No. DuPont HLO 247-96, Project No. CHW 6129-193, 28 May 1996) and "Amended Final Report (Revision No. 1), (^{14}C-PA)DPX-JE874: Determination in Plasma and Investigation of the Number and Nature of Radiolabelled Metabolites in Excreta and Selected Tissues from Male Beagle Dogs", (R. Harrison, Covance, North Yorkshire, England, DuPont Report No. HLO-1997-00761, CLE Report No. 550/29-1006, 2 April 1998). Six male Beagle dogs received a single oral gavage dose of [^{14}C-PA]DPX-JE874 at 15 mg/kg. 3 animals (Group A) were used for pharmacokinetic sampling (sacrificed at 96 hours post-dose), 3 others (Group B) were used for tissue distribution evaluation at the peak plasma concentrations

(sacrificed 2 hours post-dose) observed in Group A. The final one was used as vehicle control (Group C) (sacrificed at 96 hours). The mean recovery of radioactivity from Group A dogs during 96 hours post-dosing was 7.67% in urine, 70.3% in feces, and 0.74% in cage wash and cage wipes. Peak mean excretion of radioactivity occurred during 24 to 48 hrs post-dosing in urine and during 12-24 hours in feces. Individual animal variance was noted (See Section V, A). Group mean radioactivity concentration in plasma peaked 2 hours post-dosing at 1.53 ppm and at 4 hours in RBCs (0.626 ppm). At 96 hours, values were 0.597 ppm (plasma) and 0.648 ppm (RBCs). The highest mean concentrations of radioactivity were detected in liver (1.34 ppm) and mesenteric fat (0.945 ppm) at the 96-hour sacrifice. Mean levels in aqueous humor, eye, and eye remainder were 0.091 ppm, 0.135 ppm, and 0.173 ppm, respectively. In Group B animals (2-hour sacrifice), the highest mean concentrations of radioactivity were found in liver (4.45 ppm), mesenteric fat (2.80 ppm), plasma (0.999 ppm), and RBCs (0.413 ppm). Residues in the aqueous humor, eye, and eye remainder were 0.061 ppm, 0.106 ppm, and 0.131 ppm, respectively. DPX-JE874 and metabolites KZ007, ML815, and JL856 were identified in red blood cell and plasma extracts of Group B animals and in plasma of Group A animals. DPX-JE874 and KZ007 were identified as the prominent components in liver extracts and DPX-JE874 was the main component in fat extracts of these animals. Low concentrations of radioactivity and the small sample size precluded identification of components in the aqueous humor of the eye in Groups A and B. No identifiable components/metabolites were detected in urine samples of Group A animals. DPX-JE874, KZ007, ML815, JL856, KZ532, and KZ534 were identified in feces of Group A animals. Structures (schematic), concentration/time profiles, and proposed metabolic pathway (schematic) were included in the report (Record 207419). Acceptable. (Green and Gee, 2/25/04).

SUBCHRONIC TOXICITY

**52960-0035 207336 MacKenzie, S. A., “Subchronic oral toxicity: 90-day study with DPX-JE874-65: Feeding study in rats,” Haskell Laboratory, Newark, DE, 1/13/95. Laboratory Study #: DuPont HLR 123-93. Ten CRL:CD®BR rats/sex/group were dosed in diet with 0, 50, 200, 800, or 1600 ppm famoxadone (97.9% purity) for 90 days in the subchronic study. Estimated mean exposures were 3.3, 13, 52, and 106 mg/kg/day for males, and 4.2, 17, 66, and 130 mg/kg/day for females. Two additional groups of 5/sex/group were dosed for 2 weeks prior to sacrifice for further examinations of liver tissue responses. Cell proliferation was measured in rats which had been pre-treated with BrdU for 3 days by osmotic pumps prior to sacrifice, to assess proliferation in the liver. The other 5/sex/group were used for biochemical examinations of liver homogenates. The peroxisomal and microsomal fractions were separated by centrifugation. Peroxisomal fractions were assessed for β-oxidation, whereas cytochrome P-450 content was measured in microsomal fractions. NOEL = 50 ppm (indications of mild hemolysis, particularly decreased RBC counts and Hb concentration in both sexes at 200 ppm, also there was a small reduction in body weight gain in females). Hemolysis indications were more intense at 800 to 1600 ppm, including increased decrements in RBC counts, Hb concentration, and HCT, with associated increased reticulocyte counts in both sexes; increased serum bilirubin; generally increased urinary urobilinogen; increased spleen weights; and microscopic changes including hyperplasia of the bone marrow, and congestion, increased EMH, and increased hemosiderin in the spleen. Generalized toxicity at 800 to 1600 ppm was evident in body weight decrements (7% and 16%* in respective male groups, and 13%* and 15%* in females: * = significant, $p < 0.05$). Liver toxicity was evident by meaningful (usually statistically significant) increases at 800 and 1600 ppm of centrilobular hypertrophy, increased mitotic figures, and apoptosis (all at both dose levels in both sexes), plus focal degeneration (primarily males), and bile duct hyperplasia (only males). Liver cell proliferation was clearly demonstrated at 800 to 1600 ppm in males and in 1600 ppm females, with a non-statistically significant increase suggesting a response in 800 ppm females also. Peroxisomal β-oxidation was approximately doubled at 800 and 1600 ppm in both sexes, suggesting major functional changes at these levels. Cytochrome P-450 concentration was

unchanged. The primary clinical chemistry indicators of liver toxicity were markedly elevated circulating enzymes in 800 to 1600 ppm males (alkaline phosphatase, alanine aminotransferase, asparate aminotransferase, and sorbitol dehydrogenase). The latter was also significantly elevated in 800 to 1600 ppm females. In general, histopathology and clinical chemistry findings showed males to be preferentially affected in the liver. The study is acceptable, with no adverse effects. Aldous, 2/24/04.

52960-0036 207337 Investigators in the above subchronic study [52960-0035 207336] had considered the LOEL for body weight decrements in females to be 200 ppm. The present record refutes that determination, and places the NOEL for female body weight effects at 200 ppm, citing other rat studies of medium or long term for support. Since the original DPR review of the referenced subchronic study (above) had already placed the NOEL for female body weight effects at 200 ppm (considering the results of the combined study), there is no need for a worksheet on this supplementary record. Aldous, Jan. 5, 2004.

52960-0039 207340 This supplementary 4-week study was conducted after the above subchronic study, but before the combined rat study. It was conducted with closely-spaced dose levels of 0, 100, 200, 300, 400, 500, and 600 ppm, with the intent of placing the high dose level for the combined study in a range which would be justifiable and sustainable over the course of a lifetime study. This study assessed only body weights (which were minor and/or transitory at 500 to 600 ppm), clinical observations (which were unremarkable), and clinical chemistry parameters relating to liver (enzyme activities of alkaline phosphatase, alanine aminotransferase, asparate aminotransferase, sorbitol dehydrogenase, and γ-glutamyl transferase, plus triglyceride levels). Clinical chemistry was assessed after 2 weeks and 4 weeks. Most clinical chemistry parameters were affected at about 400 ppm. Sorbitol dehydrogenase activity in males appears to have been meaningfully elevated at 300 ppm and above at 4 weeks, and was the most sensitive indicator of liver toxicity. This study helps to justify dose levels selected for the lifetime study (DPR Record No. 207349, the “combined” study, above). Since the present study is very limited in scope, and appears unimportant for setting key endpoints, no worksheet is prepared. Aldous, Jan. 5, 2004.

****52960-0038 207339** Tompkins, E. C., “Subchronic oral toxicity: 90-day study with DPX-JE874-221: feeding study in dogs,” WIL Research Laboratories, Inc., 7/27/95. Laboratory Study #: DuPont HLO 500-94, Project No. WIL-189010. Four beagle dogs/sex/group were dosed in diet with Famoxadone at 0, 40, or 300 ppm for 13 weeks in a standard subchronic study. Also, 4/sex were initially administered 1000 ppm until marked body weight losses (or reduced gains), marked food consumption decrements, and frequent myotonic twitches prompted a dose reduction for these dogs to 600 ppm from day 37 until the end of the study. NOEL = 40 ppm for males, but undetermined for females, primarily due to a single case of degeneration in the posterior lens at 40 ppm. This female had unilateral lens fiber swelling and formation of Morgagnian globules. Such changes appeared to contrast only in degree from those which could be visualized as cataracts. Similar lesions, often either bilateral or unilateral with fiber irregularities in the contralateral eye, were observed in most 300 ppm and 600 ppm males and females. Cataracts were observed in three 300 ppm dogs, and in four 600 ppm dogs. All dogs with cataracts also had posterior lens fiber swelling and formation of Morgagnian globules at histopathology. These were the most definitive changes associated with treatment. Antecedent fiber irregularities in the posterior lens and dense body increases in the posterior or anterior lens were observed in 300 to 600 ppm dogs. In addition to the above ocular toxicity, equivocal indications of red blood cell toxicity were noted at 40 ppm and above in females, but only at 600 ppm and above in males. Findings in 40 and 300 ppm females included reduced RBC counts, reduced Hb, and reduced HCT. These findings were small in degree and showed no dose-response in that range. There was consistent evidence of RBC toxicity at 600 to 1000 ppm in both sexes (reduced RBC counts, reduced Hb, reduced HCT, increased reticulocyte and Heinz body counts, elevated platelet counts, pigmented Kupffer cells and pigmented bone marrow). The above hematology data can be compared with that of the subsequent chronic dog study (DPR Document No. 52960-0043,

Lab. Study No. DuPont HLO 820-95), which found a slight increase in platelet counts as the only hematology finding at 300 ppm, but provided ample evidence of hemolytic response at 600 ppm. The overall evidence of a meaningful hematology change in 40 ppm subchronic study females is weak. Other findings in the subchronic study at 600 ppm were myoclonic twitches (which continued after dose reduction from 1000 ppm, and which possibly resulted from markedly elevated extracellular potassium), low-weight testes and epididymides associated with immature seminiferous tubules and epididymal hypospermia, and increased kidney tubular dilatation and crystalline body deposition. Study is **acceptable**. Lens lesions are "possible adverse effects." The cited chronic dog study, which included extensive ophthalmoscopy and which confirmed lenticular degeneration at 300-600 ppm, found no ophthalmoscopic nor microscopic evidence of toxicity to the eye at 10, 20, or 40 ppm. Aldous, 3/2/04.

The cited subchronic study above reported cataracts at 300 and 600 ppm after 3 months (the time frame within which posterior cataracts generally became manifest in susceptible dogs in the chronic study also). At the next lower dose in the subchronic study (40 ppm), there were no changes at ophthalmoscopy, however a unilateral "mild" injury to the posterior lens of one 40 ppm female indicated an incipient cataract at histopathology: fiber swelling and Morgagnian globule formation. Since these posterior lens cataracts are unusual, and since there clearly was a treatment effect at higher dose levels after 3 months of exposure in both the subchronic and chronic studies, the finding at 40 ppm in the subchronic study was considered as a plausible treatment effect, evidently at the very low end of the response range. The lack of cataracts in the chronic study at 20 to 40 ppm, and also the regression of several posterior subcapsular cataracts in several 300 ppm/recovery dogs in that study, should be considered in evaluating chronic and subchronic effects of this compound.

52960-0040 207341 Supplemental to Document # 52960-0045, Record # 207347. MacKenzie, S. A., "Pilot studies with DPX-JE874-133 in mice: effects on clinical chemistry and biochemistry parameters," Haskell Laboratory, Newark, DE, 4/18/96. Laboratory Study # DuPont HLR 244-96. Groups of ten CRL:CD1®(ICR)BR mice/sex/group were treated with 0 or 3500 ppm famoxadone (Study 001) or with 0, 100, 500, 1000, 2000, 2500, or 3000 ppm famoxadone (Study 002) prior to assaying serum levels of five liver-associated enzymes after 14 days of treatment (both studies) or 28 days (Study 002 only). Triglycerides were additionally assayed in Study 002 at both intervals. After 28-31 days of treatment in Study 002, mice were sacrificed to assess liver weights. Further, liver homogenates were assayed for peroxisomal β-oxidation activity assessments and for microsomal cytochrome P-450 content. There was no NOEL found for cytochrome P-450 induction, which was substantial at all levels tested (100 to 3000 ppm). Liver weights were elevated at 500 ppm and above. An absolute NOEL for activities of liver cytosolic enzymes (ALT and SDH) and for one enzyme associated with absorptive or secretory components (ALP) was 100 ppm, based on statistically significant elevations at 500 ppm (most commonly in females). Investigators considered the NOEL for plausible indication of hepatotoxicity to be 500 ppm, based partly on the lack of concordance between activities of the several liver leakage enzymes, however the report did not provide individual data or associated analyses to support such a conclusion. Peroxisomal β-oxidation activity was elevated with LOEL's of 1000 ppm in males and 2500 ppm in females. There were no clinical signs nor body weight effects at any dose tested. This study validates that dose levels used in the mouse oncogenicity study were within an effective range to elicit liver toxicity. This study was not designed to address QA or GLP guidelines, but provides useful supplementary data. Aldous, 2/27/04.

52960-0037 207338 Biegel, L. B., "Subchronic oral toxicity: 90-day study with DPX-JE874-65: Feeding study in mice," Haskell Laboratory, Newark, DE, 11/29/94. Laboratory Study #: DuPont HLR 73-93. Ten CRL:CD1®BR mice/sex were dosed in diet for about 90 days with famoxadone (97.4% purity) at dose levels of 0, 35, 350, 3500, and 7000 ppm in a subchronic range-finding study. Estimated mean achieved doses were 0, 5.9, 62, 534, and 1149 mg/kg/day (M) and 0, 8.2, 80, 757, and 1552 mg/kg/day (F). Additional mice assigned at the same dose levels included 5/sex/group for liver cell proliferation evaluations by immunohistochemical methods following 3

days of pre-treatment with BrdU. These assessments were performed after 14-15 days of treatment. Another 5/sex/group were designated for biochemical examinations of liver homogenates after 14-15 days of treatment to assay peroxisomal fractions for β-oxidation activity and microsomal fractions for cytochrome P-450 content, respectively. NOEL = 35 ppm, based on increases in cytochrome P-450 content in livers and increased absolute and relative liver weights in females, and on increased incidence of centrilobular hypertrophy in 4/10 males at 350 ppm. These effects are considered to be reversible metabolic changes. The above findings were prominent in both sexes at 3500 and 7000 ppm. Also at those dose levels, there were significantly increased reticulocyte counts as the primary evidence of hemolysis, along with increased hemosiderin in spleens, generally increased spleen weights, and increased bile pigment in liver. Hepatocyte apoptosis and focal necrosis, usually of low incidence, were limited entirely to livers of 3500 and 7000 ppm mice, and are considered to demonstrate hepatotoxicity. Diffuse fatty change in liver was significantly elevated in 3500 to 7000 ppm males and non-significantly elevated in 7000 ppm females. Increased splenic red pulp was observed: statistically significant in 3500 to 7000 ppm females and non-significant in 7000 ppm males. Consistently increased blood hemoglobin and associated increased MCHC at 7000 ppm, often extending to 3500 ppm, further indicated hemolysis and compensation. This is a useful supplementary study, suitable to set dose levels for the oncogenicity study. Other than characterizing known or expected high dose effects of this active ingredient, no unique findings were made in this study. No adverse effects were indicated. Aldous, 2/26/04.